Thrombophilia

Introduction

This lesson dives even deeper into the clotting cascade and its regulation, introducing additional regulation factors that were left out of the initial introduction in Clotting: #1: *Hemostasis*. The emphasis here is on the balance of procoagulation factors (the clotting cascade) and anticoagulation factors (antithrombin, protein C, protein S, and plasmin). While the title of the lesson is thrombophilia, a pathologic state of hypercoagulability, the lesson is a mix of deeper physiology and the pathologic conditions that lead to the formation of extra clots.

Clotting Cascade and Regulation

There are three pathways: the platelet-activated intrinsic pathway, the endothelial cell-activated extrinsic pathway, and the common pathway. The end result of secondary hemostasis, of the clotting cascade, is the generation of a fibrin polymer, a fibrin thrombus. The common pathway is 1 five in 5, 2 fives in 10 (1, 2, 5, 10); the extrinsic pathway is exclusive (7), and the intrinsic pathway is everything else up to 12 (8, 9, 11, 12). You learned that in the last lesson. Now we add on regulation. We'll first follow the regulation of each pathway, then step back and look at the whole system, identifying which mechanisms are procoagulation and which are anticoagulation.

All mechanisms are proteolytic cleavage. That means the initial unactivated clotting factor must be cleaved to become the activated form and the activated form's getting cleaved results in an inactivated clotting factor. Cleave once to activate, cleave again to inactivate. An **un**activated clotting factor is one that has never been activated, while an **in**activated factor has been activated but is now inactive.

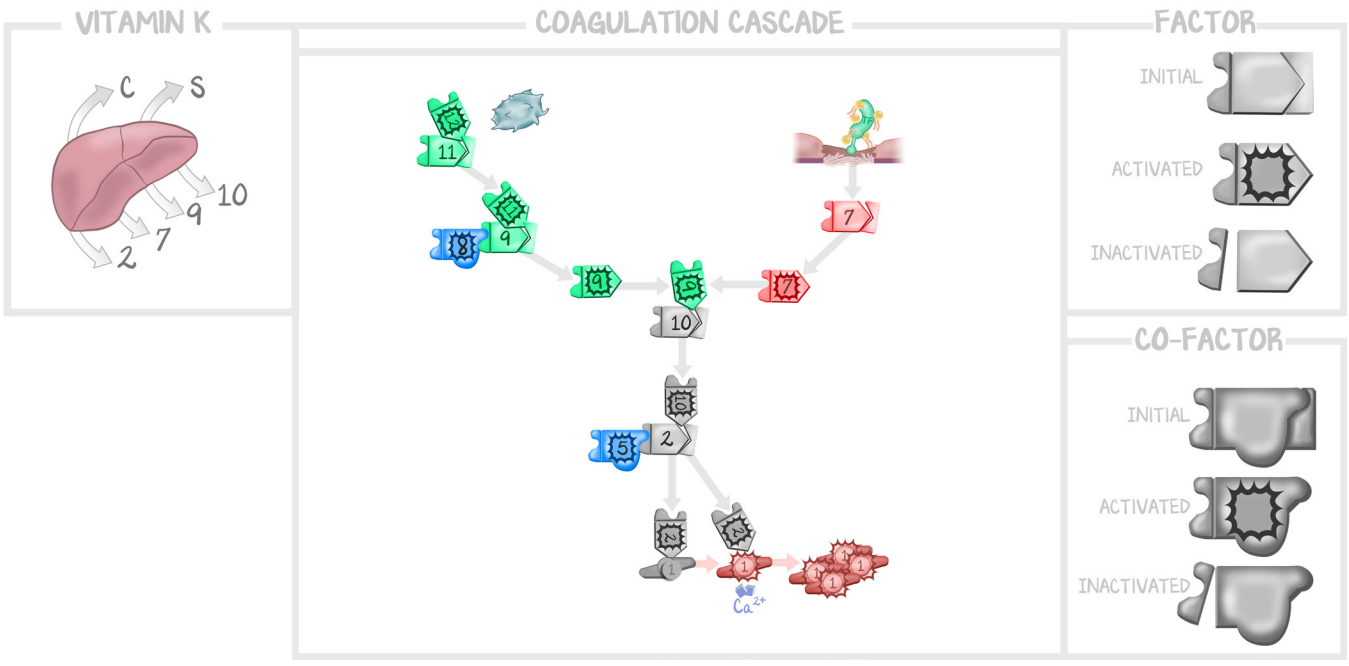

Figure 2.1: The Clotting Cascade
This is a visualization of the coming text. Refer to it as you read through until Table 2.1.

The **intrinsic pathway** is regulated at the level of factor 8. Factor 8 is **activated by factor 2** (which is thrombin) in the common pathway. The activation of factor 8 is **inhibited by protein C**. Thrombin (activated factor 2) cleaves factor 8 to form activated factor 8. Protein C cleaves activated factor 8 into a silent molecule. The intrinsic pathway follows in order—factor 12 activates 11, which in turn activates 9 with activated factor 8 as a cofactor. The mechanism that starts the process by activating factor 12 is **platelet mediated**. The activation of platelets leads to the activation of the intrinsic pathway. The intrinsic pathway ends with activated factor 9 cleaving/activating factor 10.

The **extrinsic pathway** involves only the exclusive factor 7, being activated by **thromboplastin** released from **endothelial cells**. Only one factor, only one regulatory step. There is no pathologic state of hypercoagulability that involves factor 7 or the extrinsic pathway. Interestingly, warfarin, an anticoagulant that works by inhibiting factors 2, 7, 9, and 10 at the level of the liver, has the majority of its effects felt on factor 7. Until the warfarin concentration becomes quite elevated, only the PT changes, despite 2 and 10 being part of the common pathway, and 9 being part of the intrinsic pathway. We mention this now because we need you to see it again and again. The extrinsic pathway ends with activated factor 7 cleaving/activating factor 10.

The **common pathway** starts with activated factor 10. Both the extrinsic and intrinsic pathways activate factor 10. Factor 10 in turn, using activated factor 5 as a cofactor, activates factor 2, cleaving the "pro-" from prothrombin (unactivated factor 2) to make thrombin (activated factor 2). Activated factor 2 cleaves the "-ogen" from fibrinogen to make fibrin. Regulation of this pathway is manifold. There is no strictly linear way to approach this, so we're going to start in the middle of the pathway. First, is regulation of factor 5. The cleavage/activation of thrombin by activated factor 10 requires activated factor 5. Activated **protein C** cleaves/inactivates activated factor 5. This is the same activated protein C that cleaves/inactivates activated factor 8. Protein C is therefore an anticoagulant. Second, is the regulation of thrombin (activated factor 2), which is responsible for cleaving/activating factor 1 (fibrinogen to fibrin), facilitating the formation of the fibrin thrombus (connecting fibrin monomers to form fibrin polymers), and also cleaving/activating factor 8. **Antithrombin**, as the name suggests, cleaves/inactivates thrombin, reducing the amount of fibrin that gets made, how well fibrin polymers form together, and also how much factor 8 gets activated. Here's where it gets really confusing. Third, is the regulation of activated factor 10. Antithrombin also cleaves/inactivates activated factor 10.

PROTEIN	FUNCTION	NOTES ON FUNCTION	HOW IT IS REGULATED
Protein C	Cleaves and inactivates factor 8 and factor 5	Requires activated protein S	N/A
Antithrombin	Cleaves and inactivates factor 10 and factor 2		N/A
Activated Factor 10	Cleaves and activates factor 2	Requires activated factor 5	Cleaved and inactivated by antithrombin
Activated Factor 5	Cofactor for cleavage and activation of factor 2		Cleaved and inactivated by activated protein C
Activated Factor 2 (thrombin)	Cleaves and activates factor 1 (fibrin) and factor 8		Cleaved and inactivated by antithrombin
Activated Factor 7	Cleaves and activates factor 10	Initiated by thromboplastin	
Activated Factor 9	Cleaves and activates factor 10	Initiated by platelets	Directly activated by active factor 8

Table 2.1

Once the fibrin is formed, there must be a mechanism for removing it. Fibrin is degraded into fibrin split products by **plasmin**. The fibrin polymer that is the fibrin thrombus is not a factor. Nor is plasmin. Plasmin degrades the polymers into split products and not back into fibrin monomers. Plasmin is activated by proteolytic cleavage from **plasmin(ogen)**, cleaving the -ogen to reveal active plasmin. This process is mediated by **tissue plasminogen activator**, secreted by endothelial cells. Use caution. Endothelial cells secrete tissue factor (thromboplastin) to initiate coagulation, and secrete tissue plasminogen activator (tPA) to break down the clot.

Now again from a different perspective—that of the regulators, not the steps that get regulated.

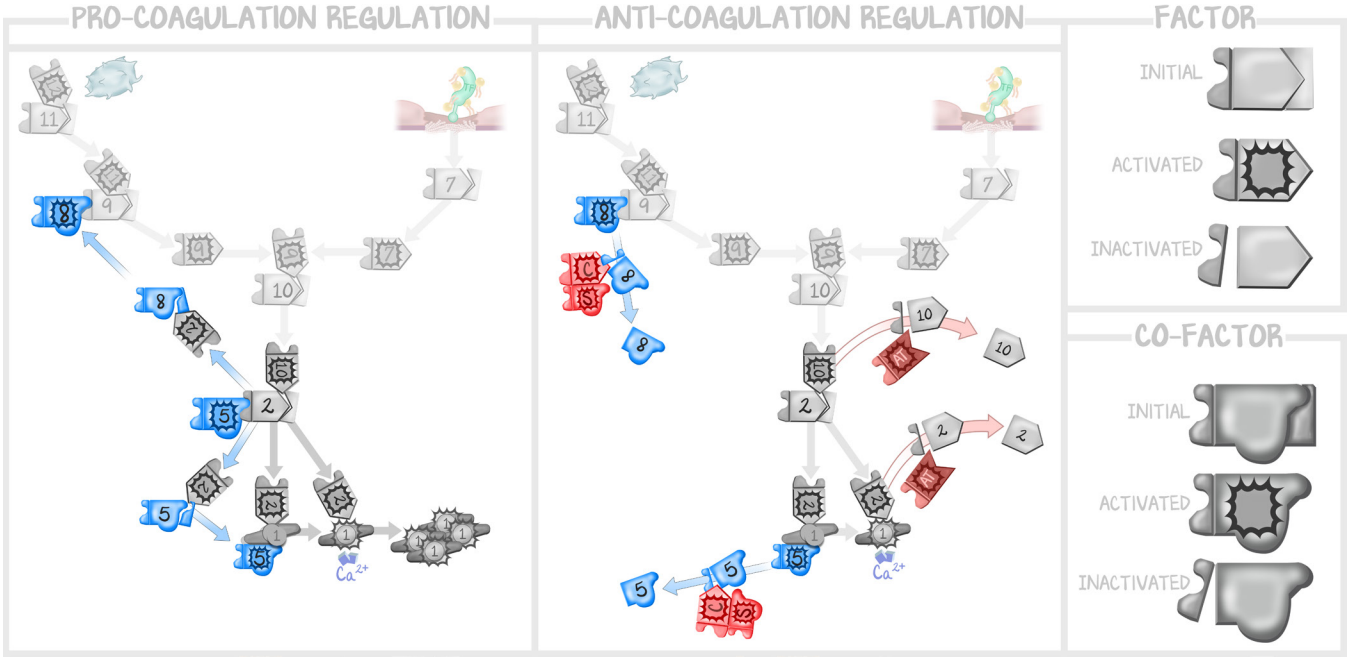

Figure 2.2: Regulation of Clotting Cascade
The visualization of the regulation of the clotting cascade as it can be divided into procoagulation (leading to clot formation by activating factors) or anticoagulation (reducing clot formation, mostly through inactivation of active factors). Use this image to visualize the coming two paragraphs.

Anticoagulants act to prevent the formation of a clot or to clear the clot. The anticoagulant molecules are protein C, protein S, antithrombin, thrombomodulin, tPA, and plasmin. **Activated protein C** cleaves and inactivates factor 8 (intrinsic) and factor 5 (common). Protein C is synthesized in the liver, as is protein S. The anticoagulant molecule on the endothelial cell surface, **thrombomodulin**, activates protein C. The inactivation of factors 8 and 5 requires **protein S as a cofactor** for activated protein C. Thrombomodulin, protein S, and protein C prevent clot formation. **Plasmin** breaks an already present fibrin thrombus into split products. tPA activates plasmin from plasminogen and is also released from endothelial cells.

Procoagulants act to facilitate formation of the clot. The procoagulant regulatory mechanisms are activated factor 8, activated factor 5, activated factor 2, and thromboplastin. **Factor 8** activates factor 9 and is activated by factor 2. **Factor 5** is required as a cofactor for factor 10 to activate factor 2. Factor 5 is cleaved and inhibited by protein C. **Factor 2** is thrombin, the molecule required for fibrin formation, fibrin polymer formation, and activation of factor 8 and factor 5. **Thromboplastin** is released from endothelial cells to initiate the extrinsic pathway. Use caution: thromboplastin from endothelial cells induces clot formation, while thrombomodulin from endothelial cells turns that induction off.

Factor Bleeding vs. Platelet Bleeding; Factor Thrombosis vs. Platelet Thrombosis

We want you to start saying, "arterial platelet clot" and "venous factor thrombosis." Both are clots and both are thrombi. Separating them like this, using different words (clot, thrombus) to make them separate, discrete entities, sets you up for success in the next lesson on pharmacology and also in the lessons on bleeding.

Arterial platelet clots occur because of high stress on the endothelium (high pressure from the heartbeat), and almost always overlie atherosclerosis. This is a high-pressure, high-flow system, and stasis cannot contribute to the development of the thrombus. Instead, propulsion of blood through the arterial system injures the endothelium. This results in platelet-rich **white thrombi** ("thrombus" is the proper term; you should start saying "clot" for arterial platelet clots). Arterial clots tend to be occlusive, causing ischemic damage to the organ distal to the occlusion. Arterial clots are treated with **antiplatelets**; antiplatelets are how we treat vascular disease—stroke, heart attack, and peripheral vascular disease. Anticoagulation, drugs that target the clotting cascade, are not used to treat arterial clots or vascular disease. Antiplatelet drugs or platelet diseases result in platelet bleeding. Platelet bleeding is generally in the form of superficial microhemorrhages that present with petechiae, gingival bleeding, or heavy menses—mucosal surfaces and skin.

Conversely, **venous factor thrombi** are found in the low-flow vessels of the veins. Because they are in low-pressure vessels with low flow through the system, these clots tend to form because of **stasis**. The absence of movement of blood allows clotting factors to activate spontaneously. Because they are in the veins and are a product of stasis, these clots tend to be larger and contain more red blood cells than arterial clots, and so are fibrin-rich **red thrombi**. They tend to mold to the shape of the vessel they form in, and are longer and larger than platelet clots. These are the thrombi that form because of immobility: postoperative and prolonged car rides we teach in the Pulmonary Module. Anticoagulation, antifactor clotting medications are used to treat deep vein thrombosis. Antiplatelets are not used to treat venous thrombi. Anticoagulation drugs or clotting cascade diseases result in factor bleeding. Factor bleeding is generally from deep macrohemorrhages, presenting with hematomas and hemarthrosis.

The breakdown of this clear delineation occurs at the level of the heart. **Mural** thrombi of the chamber wall, **vegetations** on prosthetic heart valves, and thrombi of **aortic aneurysms** are fibrin clots. Arterial platelet clots are fibrin clots. Venous factor thrombi are fibrin clots. Cardiac fibrin clots are treated with anticoagulation, even though the aorta and left ventricle are arterial. In truth, the arterial clot and the venous stasis thrombosis represent the same pathogenesis—platelet plug and fibrin thrombus—but separating them mentally now saves a huge mental burden later in training. Just remember the heart is the bridge between the two systems, and needs to be treated as its own thing, and not as "an artery."

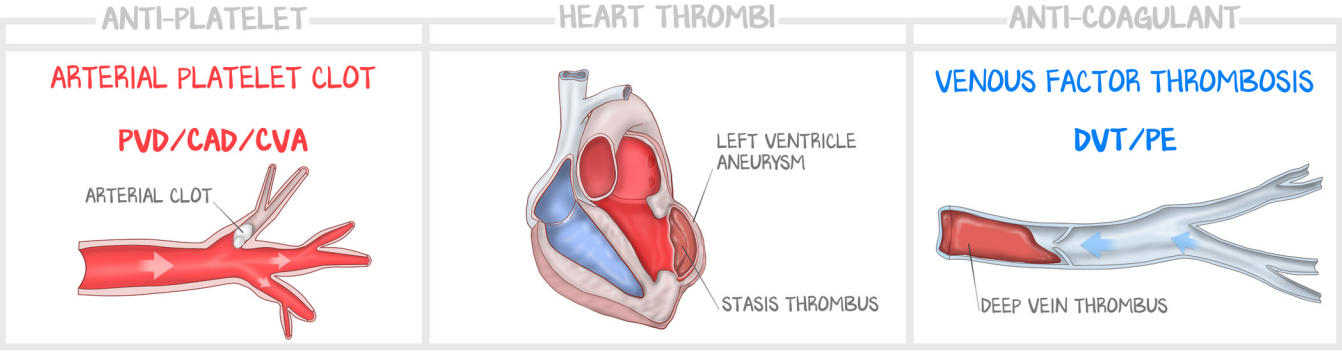

Figure 2.3: Antiplatelets vs. Anticoagulants
Antiplatelets are used to treat arterial platelet clots—PVD, CAD, CVA—while anticoagulants are used to treat venous factor thrombi.

Inherited Thrombophilia

Thrombo (fibrin thrombus formation) philia (likes) implies an increased risk of clotting. There is a tip in favor of thrombus formation. Inherited means a genetic defect leads to the thrombophilia. The emphasis in this section is going to be on inherited diseases and the methods by which they are diagnosed (either by evaluating the DNA for mutation or obtaining a blood level). For each of these conditions except lupus anticoagulant, since the defect is in the clotting cascade and not platelets, the presentation is with **deep vein thrombosis** and not arterial thrombosis, and the conditions are treated with **anticoagulation**, further justifying the separation of the arterial platelet clots and venous factor thrombosis.

Factor 5 Leiden (commonly written as "factor V Leiden") is a **mutation** in the gene for factor 5 that makes it resistant to cleavage by protein C. It is caused by a **point mutation**, a change from A to G at position 506, exchanging arginine (normal) for a glutamine (mutant). It is the **most common** inherited thrombophilia. All levels of factors and proteins are normal. However, there is a small tip in favor of thrombosis. Therefore, patients with factor 5 Leiden have an increased chance of thrombosis. But factor 5 Leiden usually requires that other risk factors for thrombosis be present to thrombose, such that factor 5 Leiden does not cause thrombosis, but merely increases the chances of its happening. Risk factors for thrombosis are **Virchow's triad**—hypercoagulability (factor 5 Leiden counts), stasis (immobility), and endothelial damage (surgery, trauma, smoking). Factor 5 Leiden almost always presents as venous thrombosis. It is treated the same way as everything else—warfarin for six months for your first DVT; lifelong warfarin for any repeat offense. Prophylactic anticoagulation for those diagnosed only with the genetic defect but without a history of DVT is NOT recommended.

Prothrombin 20210A is a **mutation** in the gene that codes for factor 2, prothrombin, located in the 3'-untranslated promoter region of the gene. It induces **increased expression** of prothrombin. With more substrate present to be converted into thrombin, the patient is at an increased risk of thrombosis. Like factor 5 Leiden, the prothrombin 20210A mutation does not cause thrombosis, but causes increased hypercoagulability that can be provoked into a thrombosis. And, like factor 5 Leiden, prothrombin 20210A tends to present with venous clots, and anticoagulation administration is agnostic of the prothrombin 20210A diagnosis. Anticoagulate a DVT; anticoagulate a prothrombin 20210A mutation with a DVT; do not anticoagulate only a diagnosis of prothrombin 20210A.

Protein C deficiency and **protein S deficiency** are both exceedingly rare disorders that are also inheritable. Since both are required for the inactivation of factor 8 and factor 5, they tip in favor of coagulation, and present like factor 5 Leiden. Because the syndrome is caused by a deficient amount of protein, measuring the **levels** of protein C and protein S reveals the diagnosis. In practice, acquired protein C and S deficiency occurs with warfarin initiation (discussed in Clotting #3: *Clotting Pharmacology*) and in nephrotic syndrome.

Antithrombin III (aka "antithrombin") deficiency produces a syndrome similar to protein C deficiency and protein S deficiency, resulting from the failed inhibition of factor 10 and factor 2. Antithrombin is stimulated by heparin, and heparin is considered an indirect inhibitor of factor 10 and factor 2 because it inhibits these factors by stimulating antithrombin. In antithrombin deficiency, the diagnosis is made by obtaining **antithrombin levels** in a patient with a thrombus. However, you can rely on one piece of information to make the diagnosis on a test question. Antithrombin deficiency would not respond to heparin administration, and therefore after heparin is administered, there will be **no change in the PTT**, separating antithrombin deficiency from all others.

Acquired Thrombophilia

Unlike hereditary disorders, the pathogenesis of **acquired thrombophilia** is frequently multifactorial. Classically, it is taught as **Virchow's triad**, though it is certainly more complex. For deep vein thrombosis, there are usually three factors—stasis, hypercoagulable state, and endothelial damage. In some cases (e.g., cardiac failure or trauma), stasis or vascular injury may be most important. Hypercoagulability due to oral contraceptive use or the hyperestrogenic state of pregnancy is caused by increased hepatic synthesis of coagulation factors and reduced anticoagulant synthesis. In disseminated cancers, release of various procoagulants from tumors predisposes to thrombosis. Smoking and obesity promote hypercoagulability by unknown mechanisms. The list is long and not worth exploring here, in this context. Instead, we discuss the specific thrombotic mechanisms in the context of the thrombotic disease—arterial platelet clot in coronary artery disease, venous factor thrombus in deep vein thrombosis.

Other causes of acquired thrombophilia that also involve thrombocytopenia are discussed in the platelet pathology lesson, because they present with low platelets, such as heparin-induced thrombocytopenia (fibrin thrombus), thrombotic thrombocytopenic purpura (platelet clots), and disseminated intravascular coagulation (both).

Special Mention: Antiphospholipid Antibody

APLA causes both **arterial clots and venous thrombosis**, and is often treated with a combination of **antiplatelet** and **anticoagulation** agents. It is also the disease with the worst names—lupus anticoagulant and antiphospholipid antibody. In the lab, the **lupus anticoagulant** prolongs the PTT. Because it is an antibody, that prolongation of the PTT is not corrected in a mixing study. However, in the patient, the lupus "anticoagulant" CAUSES coagulation. The **antiphospholipid antibodies** are not antibodies to phospholipids at all. They are not antibodies to a phospholipid but rather to epitopes on proteins that are somehow unveiled by phospholipids. The actual target of these antibodies has not been elucidated.

In APLA, clots form on "both sides" of the arterial system, presenting with arterial and venous thrombosis. That means you should look out for infarction (arterial), thrombosis (venous), or embolism (either arterial or venous). APLA can be **primary**, presenting only with the hypercoagulability and no lupus, or **secondary**, presenting as a feature of the disease lupus. Surprisingly, and suggestive of our incomplete understanding of the diagnosis, is that the described antibodies are found in 5%–15% of people who are normal without any disease.

Lupus and clots means APLA. APLA is called the lupus anticoagulant because it interferes with our PTT lab test, which is prolonged. APLA causes clots in lupus. **Lupus, clots, APLA**.

Postmortem Thrombosis

When blood stops flowing is the ultimate stasis. The blood in dead bodies coagulates. In clots that form where blood was flowing—all clots of all kinds not caused by the death of the patient—there will be **lines of Zahn**. These give the macroscopic and microscopic appearance of layers, alternating white platelet/fibrin layers with red-cell-rich layers in between. The presence of lines of Zahn means that the clot being investigated occurred prior to death. The absence of lines of Zahn means the clot being investigated should be tossed out, and no longer considered, as it was formed as a result of death, and therefore could not be the cause of death.